

Dataset: Laboratory study of long-term growth in juvenile *Menidia menidia* (Atlantic silverfish) at contrasting CO₂ levels for 16 to 122 days in 2015

Project(s): Understanding the effects of acidification and hypoxia within and across generations in a coastal marine fish (HYPOA)

Abstract: These data include the total length, wet weight, and condition factor of juvenile *Menidia menidia* (Atlantic silverfish) reared at three contrasting pCO₂ levels. Experiments were performed at the University of Connecticut's Avery Point in the Rankin Laboratory in 2015. *M. menidia* are a valued model species and pCO₂ levels were chosen to represent the contemporary and potential future conditions of productive coastal habitats. These data were collected by Dr. Hannes Baumann of the University of Connecticut in support of the collaborative research project "Understanding the effects of acidification and hypoxia within and across generations in a coastal marine fish. For a complete list of measurements, refer to the supplemental document 'Field_names.pdf', and a full dataset description is included in the supplemental file 'Dataset_description.pdf'. The most current version of this dataset is available at: <http://www.bco-dmo.org/dataset/651461>

Description: Long-term *Menidia* growth at contrasting CO₂ levels.

Total length, wet weight, and condition factor of juvenile *Menidia menidia* reared at contrasting CO₂ levels.

These data are to be associated with the corresponding paper, once published:

Murray, C.S., Fuiman, L., and Baumann, H. Consequences of elevated CO₂ exposure across multiple life stages in a coastal forage fish. *ICES Journal of Marine Science (in review)*.

Acquisition **Field sampling and experimental design:** Experiments were performed at University of Connecticut's Avery Point Campus in the Rankin Laboratory, a seawater facility adjacent to eastern Long Island Sound. Ripe adult *M. menidia* were collected on 1 May 2015 from Mumford Cove (41° 19.25' N, 72° 1.09' W), a shallow embayment dominated by eelgrasses (*Zostera marina*) and open to the Long Island Sound. Adults were sampled with a 30 m × 2 m beach seine, separated by sex, transported live to our laboratory, and held for 48 hours in large aerated tanks (17 degrees celsius, ambient CO₂, no food). On the day of fertilization (3 May 2015), greater than or equal to 20 ripe individuals from each sex were strip-spawned and eggs evenly distributed onto window screens (1 mm fiberglass mesh) submerged in plastic dishes with clear seawater. Strip-spawned adults were measured for standard length (mean SL, lower 0.5 cm; females 9.7, males 8.7). Fertilized embryos quickly attach to the screens via chorionic filaments, which facilitates precise enumeration and even allotment to treatments

and replicates. Following established protocols for rearing *M. menidia* offspring (Murray et al., 2014), replicate containers (20 l) were filled with filtered (to 1 μ m) and UV sterilized seawater (31 psu) from Long Island Sound and placed in water baths (~300 l) controlled for temperature and light conditions (17 degrees celcius, 15h light:9h dark) throughout the duration of the experiment. Within 2h of fertilization, each of four replicates per treatment received exactly 200 embryos for measure early life survival, while four other replicates per treatment each received ~400 offspring for long-term rearing. Larvae hatched ~14 days post-fertilization (dpf) and were immediately provided with standardized rations of newly hatched brine shrimp nauplii *Artemia salina* (San Francisco strain, Brine Shrimp Direct) and a commercial larval powder food (first four days, Otohime Marine Weaning Diet, size A, Reed Mariculture). At 2 days post-hatch (dph), living larvae from survival replicates were counted by gently scooping small groups into replacement containers. Between 1 to 14 days post-hatch (dph), all containers were cleaned daily with partial (10%) water exchange.

At 16 dph, larvae from the survival replicates were counted and a sub-sample ($N_{\text{control}} = 37$, $N_{\text{high}} = 33$) was preserved in 10% formaldehyde/seawater solution for later total length (TL) measurements (nearest 0.01 mm) via calibrated, digital images (ImagePro Premier V9.1). All surviving larvae were transferred to larger (50 l) tubs and maintained under the previously described protocol. At 33 dph, larvae from the survival replicates were counted and then all larvae transferred to 50 l tubs fitted with screen-covered holes (1 mm mesh) to promote water exchange from a 300 l seawater bath. Due to space constraints, from 33 to 54 dph larvae from the survival replicates were pooled into a single container per CO₂ treatment. Larvae were provided rations of nauplii and supplemented with commercial powder food (Otohime B1, Reed Mariculture). At 54 dph, all juveniles from survival and grow-out replicates were counted and pooled at equal numbers into 300 l circle tanks (two tanks per treatment, ~615 fish per tank). Juveniles were provided equal rations of newly hatched nauplii and B1 commercial powder food. Tanks were siphoned for waste daily and partial water changes completed twice weekly. Additional sub-samples for length measurements (TL, nearest 0.01 mm) were made at 36 dph ($N_{\text{control}} = 20$, $N_{\text{high}} = 20$), 68 dph ($N_{\text{control}} = 20$, $N_{\text{high}} = 20$) and 100 dph ($N_{\text{control}} = 28$, $N_{\text{high}} = 28$).

At 122 dph, the experiment was terminated and all surviving juveniles were euthanized via an overdose of Tricaine-S (MS 222, Western Chemical) for preservation. While some juveniles from each treatment were immediately frozen at -80 degrees celcius for fatty acid analyses; ~75% of the samples were fixed in 10% buffered formaldehyde/seawater solution for TL ($N_{\text{control}} = 1,025$; $N_{\text{high}} =$

1,100, nearest 0.01 mm) and weight measurements ($N_{\text{control}} = 720$; $N_{\text{high}} = 786$, nearest 0.01g).

Processing CO₂ treatments and measurements: Following best practices and guidelines for

Description: OA research (Riebesell et al., 2010) we used gas proportioners (ColeParmer) to mix air with 100% CO₂ (bone dry grade) that was delivered to the bottom of each replicate rearing container via airstones. Control conditions were achieved by forcing compressed laboratory air through a series of CO₂ stripping units containing granular soda lime (AirGas), a particle filter (1 μm) and then to each replicate via airstone. Two standardized treatment levels were administered; control (CO₂ stripped air only, ~ 500 uatm CO₂, $\text{pH}_{\text{NIST}} = 8.05$) and high CO₂ conditions (air:CO₂ mix, $\sim 2,150$ uatm CO₂, $\text{pH}_{\text{NIST}} = 7.45$). These treatments represent levels commonly used in OA research and conditions experienced seasonally by *M. menidia* offspring in the wild (Murray et al., 2014). Target pH levels were monitored daily using a handheld pH probe (Orion ROSS Ultra pH/ATC Triode and Orion Star A121 pH Portable Meter, Thermo Scientific) calibrated regularly with 2-point NIST pH references. Two sets of discrete water samples were taken from each treatment (borosilicate bottles) and immediately analyzed for total alkalinity (TA) using an endpoint titration (Mettler Toledo™ G20 Potentiometric Titrator). Methodological accuracy of alkalinity titrations were verified using Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material for TA in seawater (Batch 147 = $2,231 \text{ } \mu\text{mol TA kg seawater}^{-1}$). Actual levels of CO₂ and dissolved inorganic carbon (DIC) were then calculated in CO2SYS (<http://cdiac.ornl.gov/ftp/co2sys>) based on measured TA, pH (NIST), temperature, and salinity using K1 and K2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and Dickson (1990) for KHSO₄. An overview of the carbonate chemistry is given in Table 1.

Controlled for correctness, 3 outliers removed from dataset.

DMO Notes:

- added underscores to column headers
- added underscores to species name
- changed lat and lon to decimal format
- moved tank column last
- added underscores to site name

Deployment Information

Deployment description for lab Avery Point AP Rankin

This was where the Long-term Menidia menidia growth experiments took place. The samples were collected from offshore in Mumford Cove.

Instrument Information

Instrument	Orion ROSS Ultra pH/ATC Triode
Description	handheld pH probe
Generic Instrument Name	pH Sensor
Generic Instrument Description	General term for an instrument that measures the pH or how acidic or basic a solution is.

Instrument	Orion Star A121 pH Portable Meter
Description	portable pH meter
Generic Instrument Name	pH Sensor
Generic Instrument Description	General term for an instrument that measures the pH or how acidic or basic a solution is.

Instrument	Mettler Toledo G20
Description	Potentiometric Titrator
Generic Instrument Name	Automatic titrator
Generic Instrument Description	Instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

